Systematic molecular analysis in Hemophilia A patients in a cohort from Bogotá, Colombia

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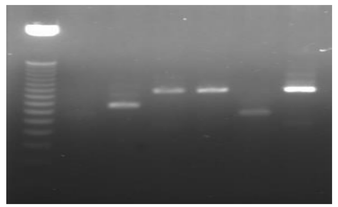
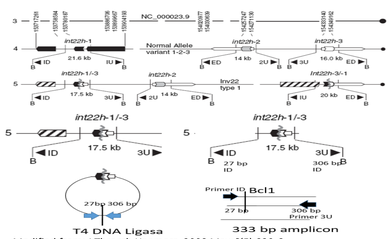
Introduction and Objectives: Hemophilia A (HA) is the most common bleeding disorder with a global incidence of 1 in 5,000 live born males. It is an X-linked recessive disorder. Worldwide, there are approximately 172,000 individuals who have the condition and of these, 60% have the severe form of the disease (plasma level of FVIII activity below 1%). Intron 22 and intron 1 inversions (Inv22 and Inv1) represent the most frequent molecular alterations found in severe HA patients with a frequency of 45-50% and 0.5-5%, respectively. Individuals with Inv22, Inv1, deletions and non-sense mutations usually have the severe form of the disease and increased risk for developing inhibitors during treatment. Here we propose a cost-effective systematic approach for the identification of molecular alterations in HA patients. **Materials and Methods:** After informed consent, we collected blood samples from 45 individuals, 37 males and 8 females. The females were 6 patient´s mothers and two patient's sisters. 30 male patients were severe, 5 moderate and 2 mild. First, for Inv22 and Inv1 testing, inverse shifting PCR was used. Patients negative for Inv22 and Inv1 were then analyzed by High Resolution Melting (HRM) for all 26 exons followed by Sanger sequencing.

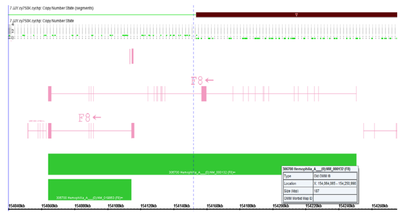


Inv22 Diagnostic test

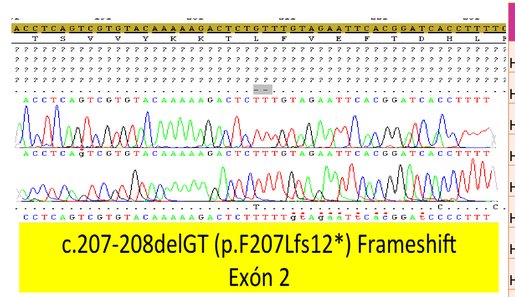


Modified from: J Thromb Haemost. 2008 May;6(5):830-6.

Schematic representation of IS-PCR

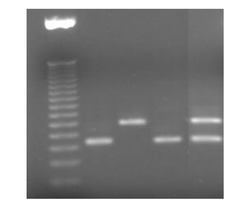


Exon 1-14 deletion Affymetrix CytoScan 750

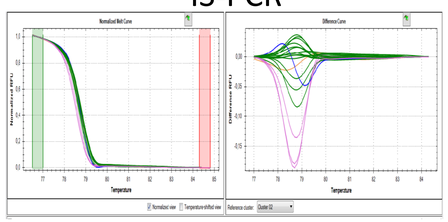


c.207-208delGT (p.F207Lfs12*) Frameshift Exón 2

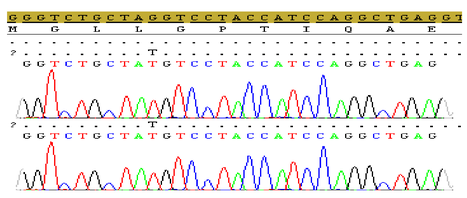
ID	Variation	Exon /Intron	Type	Reported	F8 % activity	Severity HA
HA-07	Exon 1-14 del (220 Kb)	1-14	Deletion	Yes	<1	Severe
HA-10	c.157C>T (p.V653M)	13	Missense	NO	2,0	Moderate
HA-11	Exon 26 del	26	Deletion	Yes	0,6	Severe
HA-13	22 Kb Del (exon 13)	13	Deletion	Yes	0,8	Severe
HA-17	c.1292T>C (p.L431S)	9	Missense	Yes	3,0	Moderate
HA-18	c.274G>T (p.G92C)	3	Missense	Yes	0,4	Severe
HA-20	c.5666A>G (p.Q1889R)	17	Missense	NO	12,0	Mild
HA-24	c.5953C>T (p.R1985*)	18	Non sense	YES	0,5	Severe
HA-32	c.2095A>G (M699V)	13	Missense	Yes	3.7	Moderate
HA-33	c.207-208delGT (p.F207Lfs*12)	2	Frameshift	YES	0,7	Severe
HA-37	c.262A>G (p.M88V)	2	Missense	NO	0.2	Severe
HA-40	c.1892A>G (N631S)	12	Missense	YES	3	Moderate



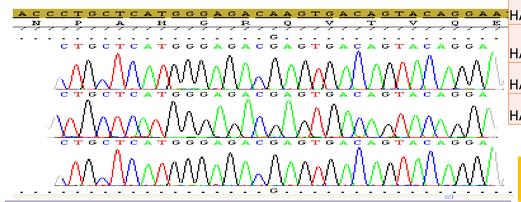
Inv1 Diagnostic test



HRM analysis



c.274G>T (p.G92C) Missense Exón 3



c.5666A>G (p.Q1889R) missense Exón 17

Variation	N	F	Severity
INVERSION 22	18/43	41.9%	Severe/Moderate (1)
INVERSION 1	4/43	9.3%	Severe
DELETION (Large)	4/43	9.3%	Severe
MISSENSE	10/43	23.3%	Severe/Moderate/Mild
NONSENSE	1/43	2.3%	Severe
FRAMESHIFT	2/43	4.7%	Severe
TOTAL	39/43	90.7%	

Results: 18/43 samples showed Inv22 (41.9%), Inv1 4/43 (9.3%) and large deletions in four patients (9.3%). Between the women included, two tested positive for Inv22 (2/8, 25%), one for Inv1 (1/8, 12%) and one for large deletion (1/8, 12%). The two sisters tested normal. Large deletions were confirmed by Affymetrix microarray analysis in 3 patients. After HRM and Sanger sequencing, we identified missense variations in 10/43 (23.3%), nonsense in 1/43 (2.3%), frameshift in 2/43 (4.7%) .

Conclusions: By this cost-effective systematic approach, we identified for the first time in Colombia, that 91% of our patients carried Inv22, Inv1, deletions or variations in coding sequence. These results are similar to results found in other populations. However, three new pathogenic variants are described, c.157C>T (p.V653M) in a moderate HA patient, c.5666A>G (p.Q1889R) in two mild patients and their mother and c.262A>G (p.M88V) in a severe HA patient. Current analysis is underway in order to identify the molecular alteration in the remaining 4/43 of patients that were negative by this approach.

Bibliography

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